

1 **Claims**

2

3 1. A method of determining the presence of a
4 toxicant in a test sample, comprising the steps
5 of;

- 6 • exposing a eukaryote that has been
7 transformed with a light emitting Ca^{2+}
8 regulated photoprotein gene to a test sample
9 • measuring the light produced by the
10 transformed cell/organism
11 • determining whether the amount of light is
12 above or below a defined threshold at the
13 time of exposure.

14

15 2. A method as in Claim 1 wherein the eukaryote is a
16 fungi.

17

18 3. A method as in Claim 2 wherein the fungi is a
19 filamentous fungi.

20

21 4. A method as in Claims 2 or 3 wherein the fungi is
22 of the *Aspergillus* species.

23

24 5. A method as in Claim 1 wherein the eukaryote is a
25 mammalian cell.

26

27 6. A method as in Claim 1 wherein the eukaryote is a
28 plant cell.

29

30 7. A method as in any of the previous Claims wherein
31 the test sample comprises a toxicant.

- 1 8. A method as in any of the previous Claims wherein
2 the light emitting Ca^{2+} regulated photoprotein
3 gene is a recombinant gene.
4
- 5 9. A method as in any of the previous Claims wherein
6 the light emitting Ca^{2+} regulated photoprotein
7 gene is selected from the group comprising;
 - 8 • aequorin gene
 - 9 • halistaurin (mitrocomin) gene
 - 10 • phialidin (clytin) gene
 - 11 • obelin gene
 - 12 • mnemiopsin gene
 - 13 • berovin gene
- 14
- 15 10. A method as in any of the previous Claims
16 wherein the light emitting Ca^{2+} regulated
17 photoprotein gene may be a functional homologue
18 of a gene selected from the group comprising;
 - 19 • aequorin gene
 - 20 • halistaurin (mitrocomin) gene
 - 21 • phialidin (clytin) gene
 - 22 • obelin gene
 - 23 • mnemiopsin gene
 - 24 • berovin gene
- 25
- 26 11. A method as in any of the previous Claims
27 wherein the light emitting Ca^{2+} regulated
28 photoprotein gene is an aequorin gene.
- 29

- 1 12. A method as in any of the previous Claims
2 wherein the light emitting Ca^{2+} regulated
3 photoprotein gene is a recombinant aequorin gene.
- 4
- 5 13. A method as in any of the previous Claims
6 wherein the light that is measured is in the form
7 of luminescence.
- 8
- 9 14. A method as in any of the previous Claims
10 wherein the test sample is added in advance of
11 the application of a stimulus to the test sample.
- 12
- 13 15. A method as in Claim 14 wherein the stimulus is
14 at least one or more from the group comprising;
15 mechanical perturbation, hypo-osmotic shock,
16 change in external calcium chloride
17 concentration, temperature shock and pH shock.
- 18
- 19 16. A method as in Claims 14 and 15 wherein the
20 test sample is added 1 minute to 1 hour prior to
21 the application of the stimulus.
- 22
- 23 17. A method as in Claims 14 to 16 wherein the test
24 sample is added 5 minutes prior to the
25 application of the stimulus.
- 26
- 27 18. A method as in Claims 14 to 16 wherein the test
28 sample is added 30 minutes prior to the
29 application of the stimulus.
- 30

- 1 19. A method of determining the presence of a
2 toxicant in a test sample, comprising the steps
3 of;
 - 4 • exposing a eukaryote that has been
5 transformed with a light emitting Ca^{2+}
6 regulated photoprotein gene to a test sample
 - 7 • measuring the light produced by the
8 transformed cell/organism
 - 9 • determining whether the amount of light is
10 above a defined threshold at a specified
11 time after the time of exposure.
- 12
- 13 20. A method as in Claim 19 which comprises the
14 step of determining whether the amount of light
15 is below a defined threshold.
- 16
- 17 21. A method as in Claims 19 and 20 wherein the
18 specified time after the time of exposure is 11
19 minutes.
- 20
- 21 22. A method as in Claims 19 to 21 wherein the
22 eukaryote is a fungi.
- 23
- 24 23. A method as in Claim 22 wherein the fungi is a
25 filamentous fungi.
- 26
- 27 24. A method as in Claims 22 to 23 wherein the
28 fungi is of the *Aspergillus* species.
- 29
- 30 25. A method as in Claims 19 to 21 wherein the
31 eukaryote is a mammalian cell.
- 32

- 1 26. A method as in Claims 19 to 21 wherein the
2 eukaryote is a plant cell.
3
- 4 27. A method as in Claims 19 to 26 wherein the test
5 sample comprises a toxicant.
6
- 7 28. A method as in Claims 19 to 27 wherein the
8 light emitting Ca^{2+} regulated photoprotein gene is
9 a recombinant gene.
10
- 11 29. A method as in Claims 19 to 28 wherein the
12 light emitting Ca^{2+} regulated photoprotein gene is
13 selected from the group comprising;
 - 14 • aequorin gene
 - 15 • halistaurin (mitrocomin) gene
 - 16 • phialidin (clytin) gene
 - 17 • obelin gene
 - 18 • mnemiopsin gene
 - 19 • berovin gene
20
- 21 30. A method as in Claims 19 to 29 wherein the
22 light emitting Ca^{2+} regulated photoprotein gene
23 may be a functional homologue of a gene selected
24 from the group comprising;
 - 25 • aequorin gene
 - 26 • halistaurin (mitrocomin) gene
 - 27 • phialidin (clytin) gene
 - 28 • obelin gene
 - 29 • mnemiopsin gene
 - 30 • berovin gene
31

1 31. A method as in Claims 19 to 30 wherein the
2 light emitting Ca^{2+} regulated photoprotein gene is
3 an aequorin gene.

4

5 32. A method as in Claims 31 wherein the light
6 emitting Ca^{2+} regulated photoprotein gene is a
7 recombinant aequorin gene.

8

9 33. A method as in Claims 19 to 32 wherein the
10 light that is measured is in the form of
11 luminescence.

12

13 34. A method as in Claims 19 to 33 wherein the test
14 sample is added in advance of the application of
15 a stimulus to the test sample.

16

17 35. A method as in Claim 34 wherein the stimulus is
18 at least one or more from the group comprising;
19 mechanical perturbation, hypo-osmotic shock,
20 change in external calcium chloride
21 concentration, temperature shock and pH shock.

22

23 36. A method as in Claims 34 to 35 wherein the test
24 sample is added 1 minute to 1 hour prior to the
25 application of the stimulus.

26

27 37. A method as in Claims 34 to 36 wherein the test
28 sample is added 5 minutes prior to the
29 application of the stimulus.

30

1 38. A method as in Claims 34 to 36 wherein the test
2 sample is added 30 minutes prior to the
3 application of the stimulus.

4

5 39. A method of determining the presence of a
6 toxicant in a test sample, comprising the steps
7 of;

- 8 • exposing a eukaryote that has been
9 transformed with a light emitting Ca^{2+}
10 regulated photoprotein gene to a test sample
- 11 • measuring the light produced by the
12 transformed cell/organism
- 13 • and comparing the light measurement data
14 with a bank of known toxicity reference
15 data.

16

17 40. A method as in Claim 39 wherein the method
18 comprises the step of determining whether the
19 amount of light is below a defined threshold.

20

21 41. A method as in Claims 39 to 40 wherein the
22 specified time after the time of exposure is 11
23 minutes.

24

25 42. A method as in Claims 39 to 40 wherein the
26 eukaryote is a fungi.

27

28 43. A method as in Claim 42 wherein the fungi is a
29 filamentous fungi.

30

31 44. A method as in Claims 42 to 43 wherein the
32 fungi is of the *Aspergillus* species.

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2 45. A method as in Claims 39 to 41 wherein the
3 eukaryote is a mammalian cell.

4

5 46. A method as in Claims 39 to 41 wherein the
6 eukaryote is a plant cell.

7

8 47. A method as in Claims 39 to 46 wherein the test
9 sample comprises a toxicant.

10

11 48. A method as in Claims 39 to 47 wherein the
12 light emitting Ca^{2+} regulated photoprotein gene is
13 a recombinant gene.

14

15 49. A method as in Claims 39 to 48 wherein the
16 light emitting Ca^{2+} regulated photoprotein gene is
17 selected from the group comprising;

- 18 • aequorin gene
- 19 • halistaurin (mitrocomin) gene
- 20 • phialidin (clytin) gene
- 21 • obelin gene
- 22 • mnemiopsis gene
- 23 • berovin gene

24

25 50. A method as in Claims 39 to 49 wherein, the
26 light emitting Ca^{2+} regulated photoprotein gene
27 may be a functional homologue of a gene selected
28 from the group comprising;

- 29 • aequorin gene
- 30 • halistaurin (mitrocomin) gene
- 31 • phialidin (clytin) gene

1 • obelin gene

2 • mnemiopsis gene

3 • berovin gene

4

5 51. A method as in Claims 39 to 50 wherein the
6 light emitting Ca^{2+} regulated photoprotein gene is
7 an aequorin gene.

8

9 52. A method as in Claims 39 to 51 wherein the
10 light emitting Ca^{2+} regulated photoprotein gene is
11 a recombinant aequorin gene.

12

13 53. A method as in Claims 39 to 52 wherein the
14 light that is measured is in the form of
15 luminescence.

16

17 54. A method as in Claims 39 to 53 wherein the test
18 sample is added in advance of the application of
19 a stimulus to the test sample.

20

21 55. A method as in Claim 54 wherein the stimulus is
22 at least one or more from the group comprising;
23 mechanical perturbation, hypo-osmotic shock,
24 change in external calcium chloride
25 concentration, temperature shock and pH shock.

26

27 56. A method as in Claims 54 to 55 wherein the test
28 sample is added 1 minute to 1 hour prior to the
29 application of the stimulus.

30

1 57. A method as in Claims 54 to 56 wherein the test
2 sample is added 5 minutes prior to the
3 application of the stimulus.

4

5 58. A method as in Claims 54 to 55 wherein the test
6 sample is added 30 minutes prior to the
7 application of the stimulus.

8

9 59. A method as in Claims 39 to 58 wherein the
10 method is used to determine the amount of
11 toxicant in the sample.

12

13 60. A method as in Claims 39 to 59 wherein the
14 method is used to identify the toxicant in the
15 sample.

16

17 61. A method of determining the presence of a
18 toxicant in a test sample, comprising the steps
19 of;

- 20 • exposing a eukaryote that has been
21 transformed with a light emitting Ca^{2+}
22 regulated photoprotein gene to a test sample
- 23 • measuring the light produced by the
24 transformed cell/organism
- 25 • converting the light data into a cytosolic
26 free calcium ion concentration trace,
- 27 • and comparing at least one parameter of the
28 cytosolic free calcium ion concentration
29 trace with a bank of known toxicity
30 reference data.

31

- 1 62. A method as in Claim 61 wherein the method
2 comprises the step of determining whether the
3 amount of light is below a defined threshold.
4
- 5 63. A method as in Claims 61 to 62 wherein the
6 eukaryote is a fungi.
7
- 8 64. A method as in Claim 63 wherein the fungi is a
9 filamentous fungi.
10
- 11 65. A method as in Claims 63 to 64 wherein the
12 fungi is of the *Aspergillus* species.
13
- 14 66. A method as in Claims 61 to 62 wherein the
15 eukaryote is a mammalian cell.
16
- 17 67. A method as in Claims 61 to 62 wherein the
18 eukaryote is a plant cell.
19
- 20 68. A method as in Claims 61 to 67 wherein the test
21 sample comprises a toxicant.
22
- 23 69. A method as in Claims 61 to 68 wherein the
24 light emitting Ca^{2+} regulated photoprotein gene is
25 a recombinant gene.
26
- 27 70. A method as in Claims 61 to 69 wherein the
28 light emitting Ca^{2+} regulated photoprotein gene is
29 selected from the group comprising;
30 • aequorin gene
31 • halistaurin (mitrocomin) gene
32 • phialidin (clytin) gene

- 1 • obelin gene
- 2 • mnemiopsis gene
- 3 • berovin gene
- 4
- 5 71. A method as in Claims 61 to 70 wherein the
- 6 light emitting Ca^{2+} regulated photoprotein gene
- 7 may be a functional homologue of a gene selected
- 8 from the group comprising;
- 9 • aequorin gene
- 10 • halistaurin (mitrocomin) gene
- 11 • phialidin (clytin) gene
- 12 • obelin gene
- 13 • mnemiopsis gene
- 14 • berovin gene
- 15
- 16 72. A method as in Claims 61 to 71 wherein the
- 17 light emitting Ca^{2+} regulated photoprotein gene is
- 18 an aequorin gene.
- 19
- 20 73. A method as in Claims 61 to 72 wherein the
- 21 light emitting Ca^{2+} regulated photoprotein gene is
- 22 a recombinant aequorin gene.
- 23
- 24 74. A method as in Claims 61 to 73 wherein the
- 25 light that is measured is in the form of
- 26 luminescence.
- 27
- 28 75. A method as in Claims 61 to 74 wherein the test
- 29 sample is added in advance of the application of
- 30 a stimulus to the test sample.
- 31

1 76. A method as in Claim 75 wherein the stimulus is
2 at least one or more from the group comprising;
3 mechanical perturbation, hypo-osmotic shock,
4 change in external calcium chloride
5 concentration, temperature shock and pH shock.

6
7 77. A method as in Claims 75 to 76 wherein the test
8 sample is added 1 minute to 1 hour prior to the
9 application of the stimulus.

10
11 78. A method as in Claims 75 to 77 wherein the test
12 sample is added 5 minutes prior to the
13 application of the stimulus.

14
15 79. A method as in Claims 75 to 77 wherein the test
16 sample is added 30 minutes prior to the
17 application of the stimulus.

18
19 80. A method as in Claims 61 to 79 wherein light is
20 measured for between 1 minute and 5 hours
21 following the application of the stimulus.

22
23 81. A method as in Claims 61 to 79 wherein light is
24 measured for between 1 minute and 96 hours
25 following the application of the stimulus.

26
27 82. A method as in Claims 61 to 79 wherein light is
28 measured for 5 minutes following the application
29 of the stimulus.

30
31 83. A method as in Claims 61 to 82 wherein the
32 cytosolic free calcium ion trace is a plot of the

1 cytosolic free calcium ion concentration against
2 time.

3

4 84. A method as in Claims 61 to 83 wherein the
5 parameter is at least one or more selected from
6 the group comprising;

- 7 • lag time
8 • rise time
9 • absolute amplitude
10 • relative amplitude
11 • Length of transient at 20%, 50% and 80% of
12 maximum amplitude
13 • number of cytosolic free calcium ion
14 concentration increases
15 • percentage increase in final cytosolic free
16 calcium ion concentration resting level
17 • percentage increase in recovery time
18 • percentage increase in pre-stimulating
19 cytosolic free calcium ion concentration
20 resting level
21 • total concentration of calcium

22

23 85. A method as in Claims 61 to 84 wherein the
24 method is used to determine the amount of
25 toxicant in the sample.

26

27 86. A method as in Claims 61 to 85 wherein the
28 method is used to identify the toxicant in the
29 sample.

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